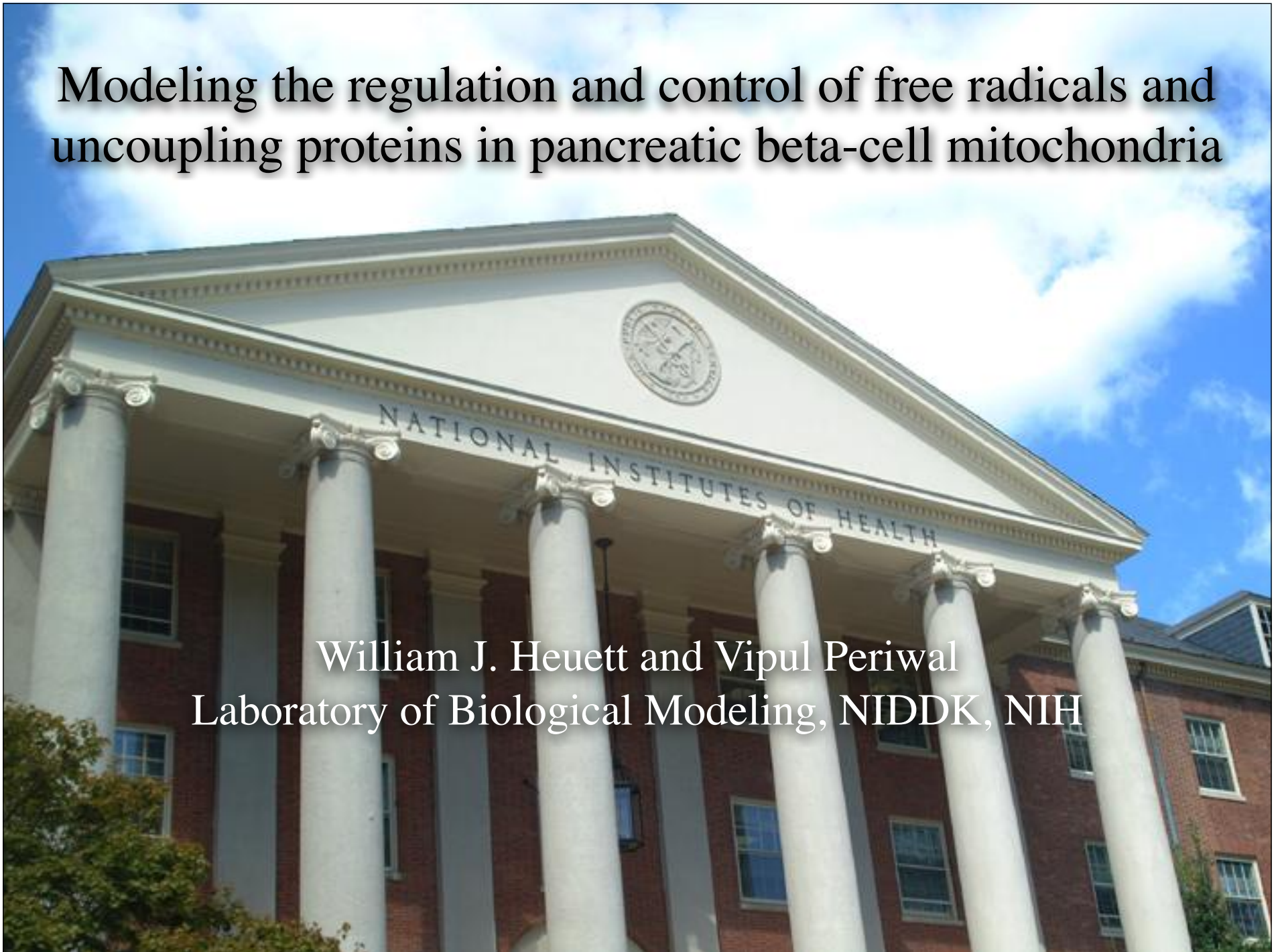


Modeling the regulation and control of free radicals and uncoupling proteins in pancreatic beta-cell mitochondria

William J. Heuett and Vipul Periwal
Laboratory of Biological Modeling, NIDDK, NIH



The Overflowing American Dinner Plate

IN 1970, the average American ate about 16.4 pounds of food a week, or 2.3 pounds daily. By 2006, the average intake grew by an additional 1.8 pounds a week.

Among other things, that's an extra half pound of fat weekly — mostly from oils and

shortening. That doesn't count the fat in the extra quarter pound of meat Americans now eat every seven days. Those fats were somewhat offset by a steep drop in dairy consumption, the only major food group to have a decline, primarily in milk drinking. (But we do love our cheese. More and more of it.)

This portrait of the raw ingredients of the

American diet is based on what the Agriculture Department calls "food availability" — the amount of food produced for the average American consumer. The data are adjusted for food losses (waste on farms; in processing and transportation; and in stores, restaurants and homes) to provide a close approximation of what individuals eat. (The most recent year for which data are available is 2006.)

The numbers don't reveal how much grain went into bread versus cookies, or how many chicken breasts became chicken nuggets. But the overall increase in eating does suggest a link with the rise in Americans' weight over the same period. According to the Centers for Disease Control, 15 percent of adults age 20 to 74 were obese by 1980. By 2007, that had more than doubled.

The Weekly Diet in 1970...

FIGURES IN APPROX. POUNDS



Photographs depict the weight and broad categories of foods consumed weekly, but not their full variety (the many different vegetables, for example).

...And How It Changed By 2006



Whole milk consumption plunged, with lower-fat milks replacing only some of that; soft drinks and bottled water are now preferred beverages.

Americans are eating more vegetables, but still not enough to meet federal recommendations.

Red meat is down; poultry is way up. Fish, growing fastest, is less than 10 percent of the category.

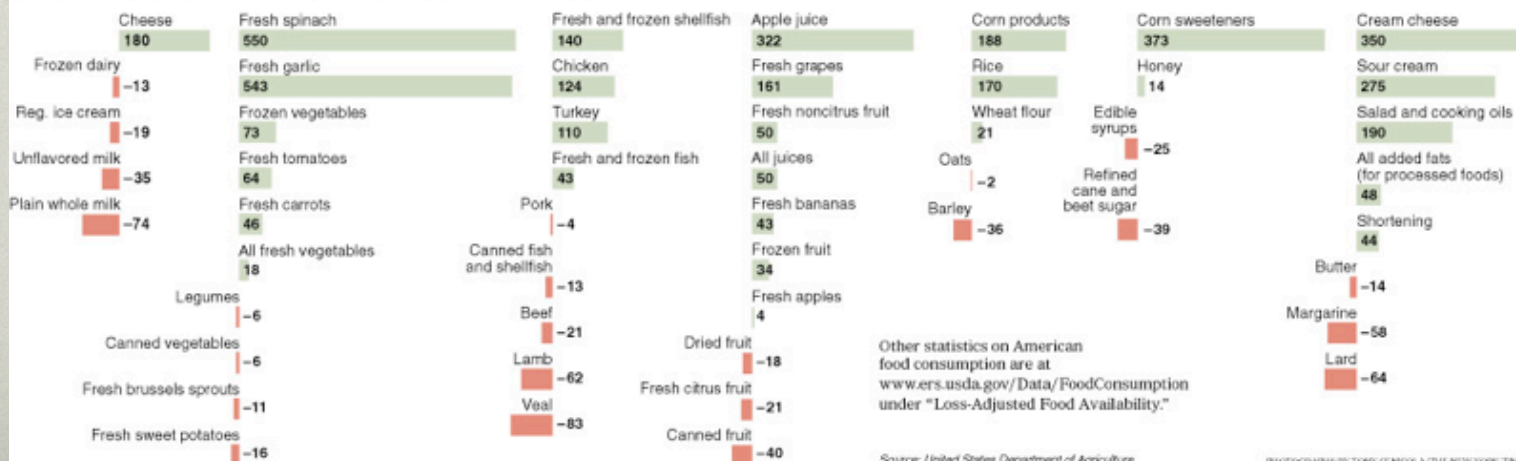
Nearly half of fruit consumption is in the form of juice, with some of that used as a food sweetener.

Almost 90 percent are refined grains; government guidelines call for far more whole grains.

This is what goes into processed foods (not sugars occurring naturally in fruit and milk).

The fastest-growing of these food categories, it includes both oils and some animal fats.

Percentage Change in Consumption of Selected Foods, 1970-2006



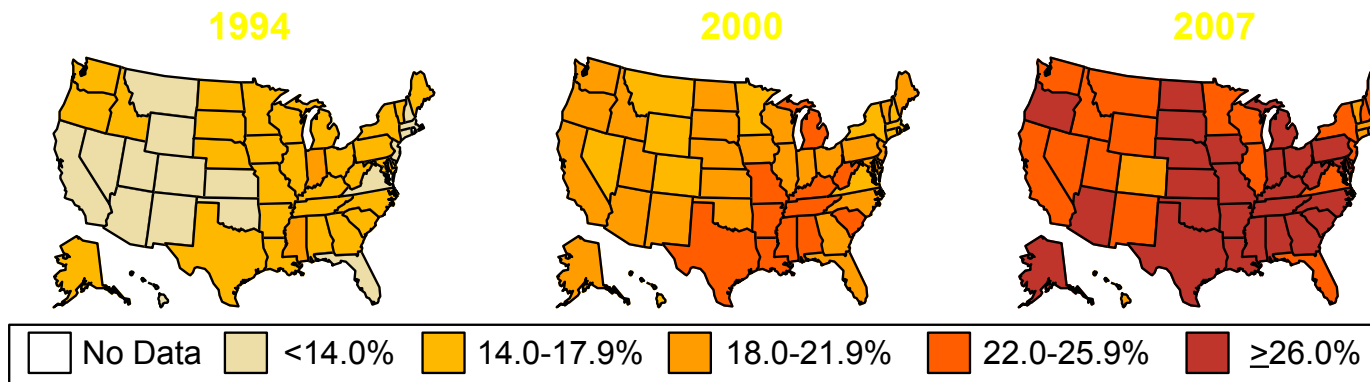
Other statistics on American food consumption are at www.ers.usda.gov/Data/FoodConsumption under "Loss-Adjusted Food Availability."

Source: United States Department of Agriculture

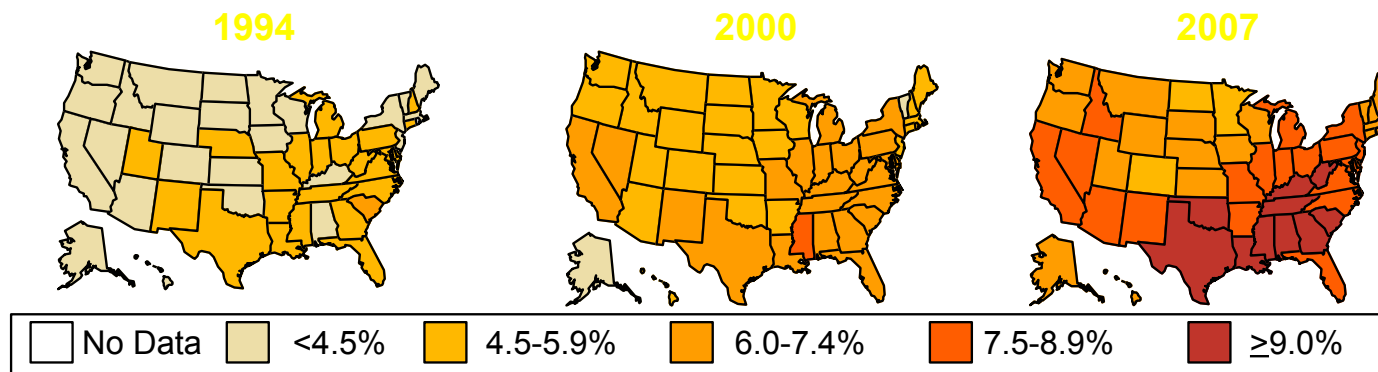
PHOTOGRAPHS BY TONY CENICOLA/THE NEW YORK TIMES

Age-adjusted Percentage of U.S. Adults Who Were Obese or Who Had Diagnosed Diabetes

Obesity (BMI ≥ 30 kg/m²)



Diabetes



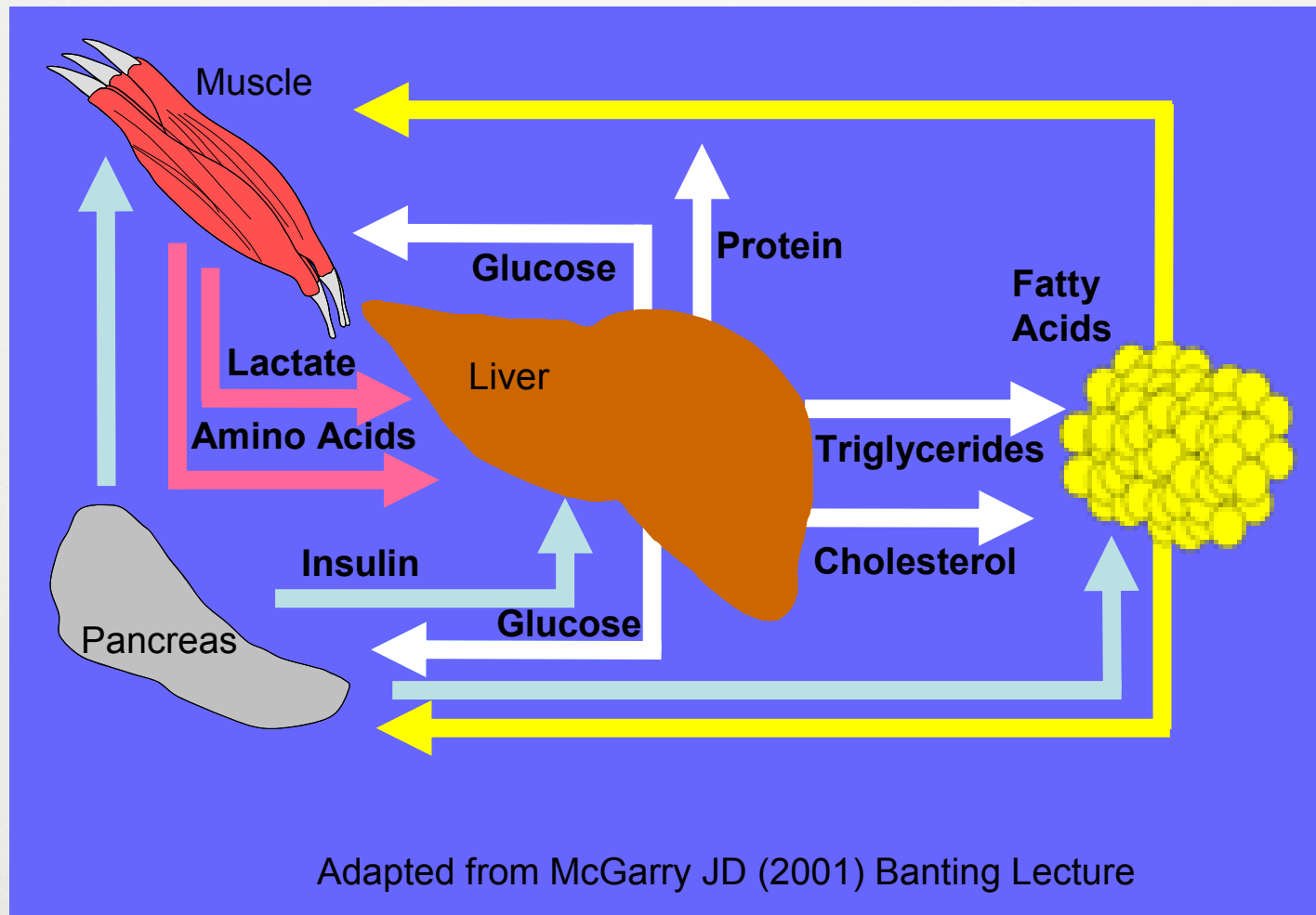
CDC's Division of Diabetes Translation. National Diabetes Surveillance System available at <http://www.cdc.gov/diabetes/statistics>



Increased Risks:

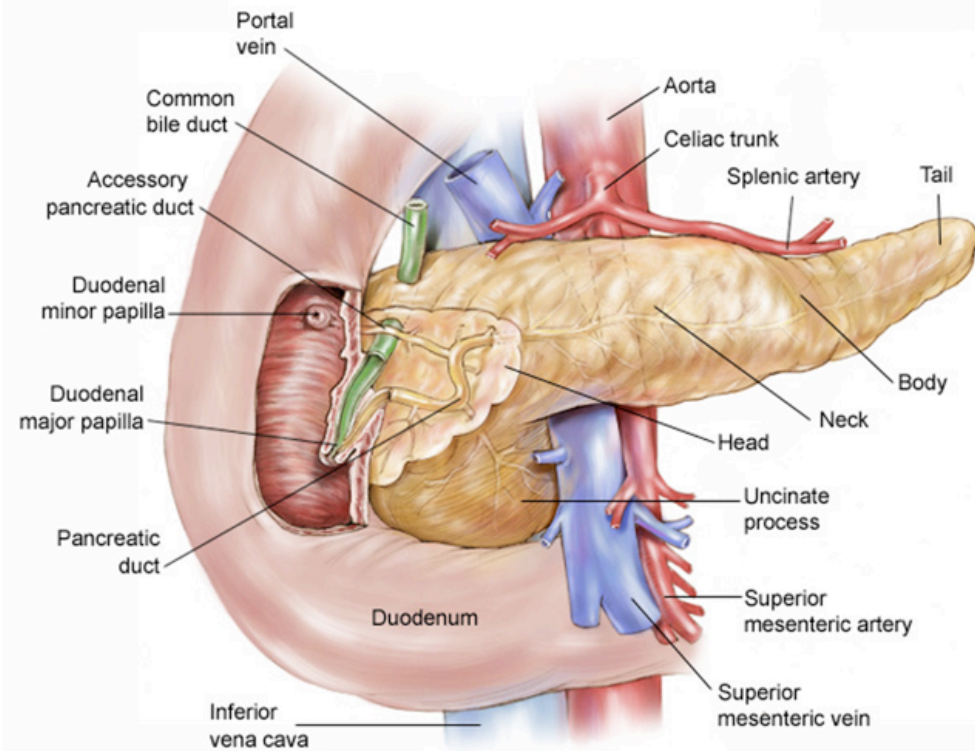
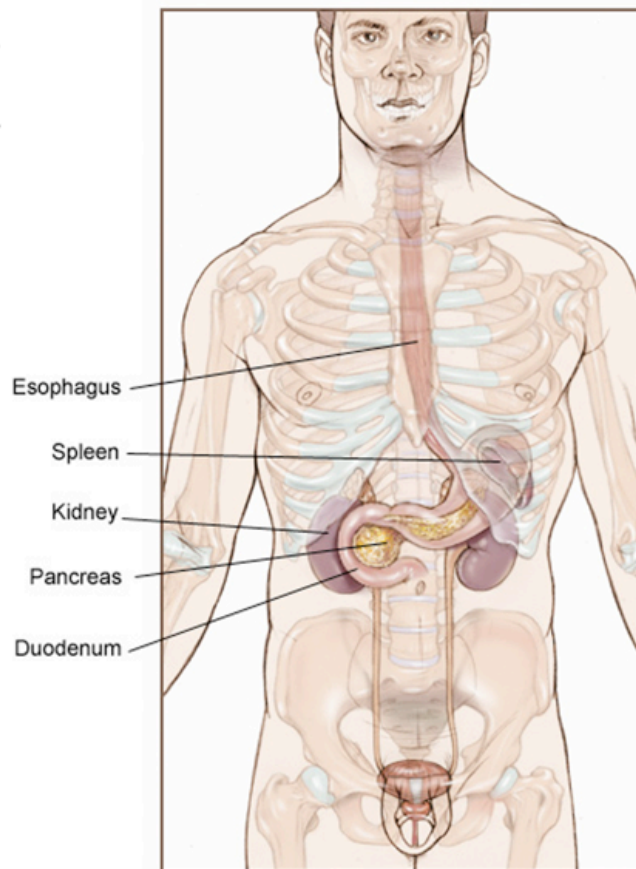
- Coronary heart disease
- Type 2 diabetes
- Cancers (endometrial, breast, and colon)
- Hypertension (high blood pressure)
- Dyslipidemia (for example, high total cholesterol or high levels of triglycerides)
- Stroke
- Liver and gallbladder disease
- Sleep apnea and respiratory problems
- Osteoarthritis (a degeneration of cartilage and its underlying bone within a joint)
- Gynecological problems (abnormal menses, infertility)

Whole Body Metabolism



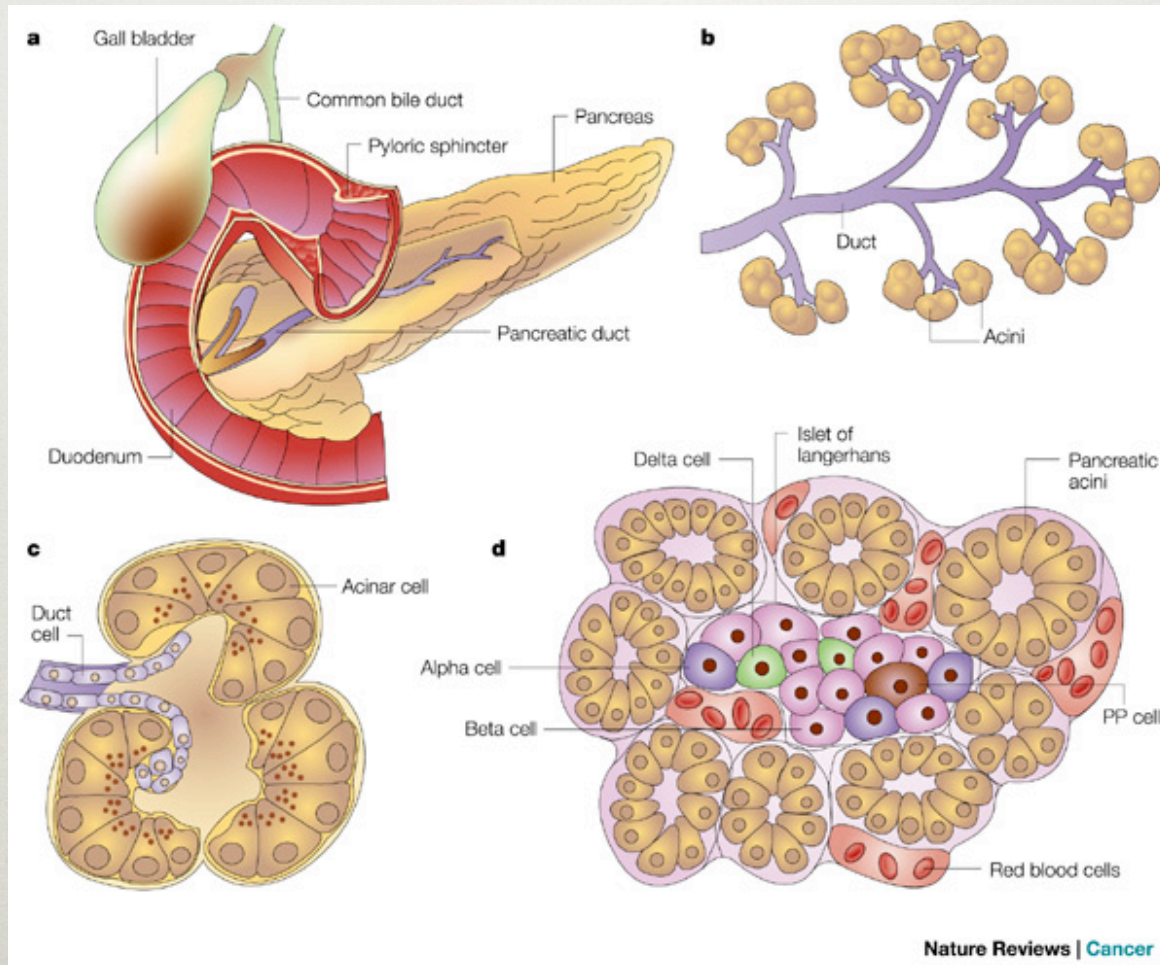
The Pancreas

Robert Morreale/Visual Explanations, LLC



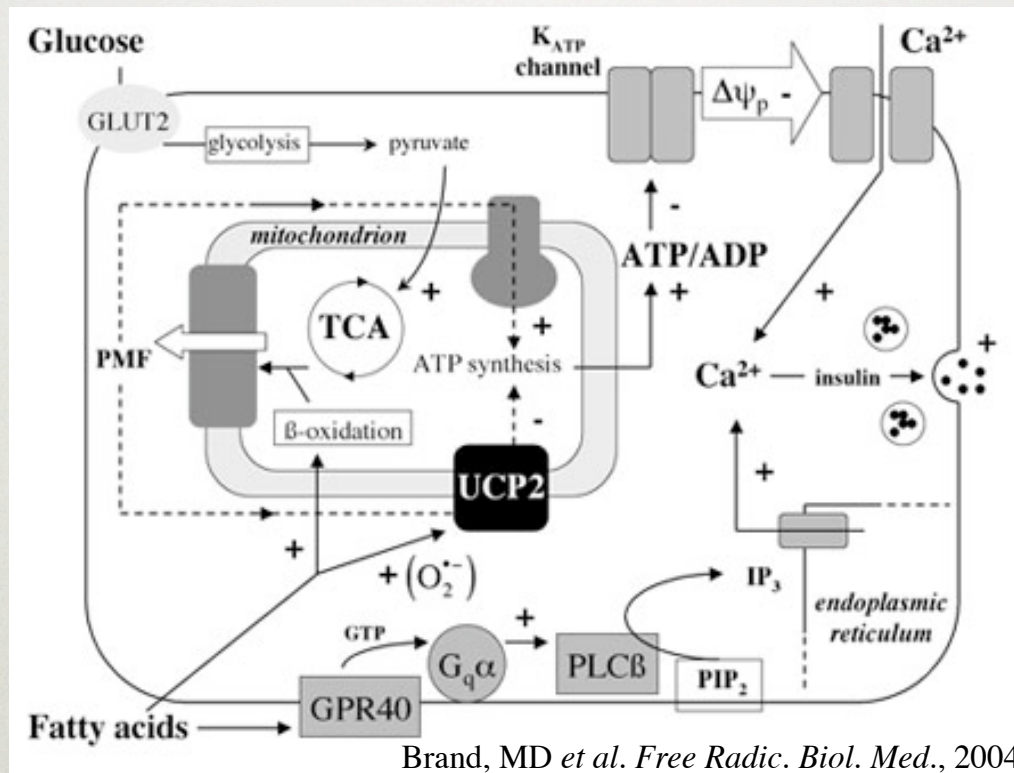
© 2004 American Society of Clinical Oncology

Islets of Langerhans

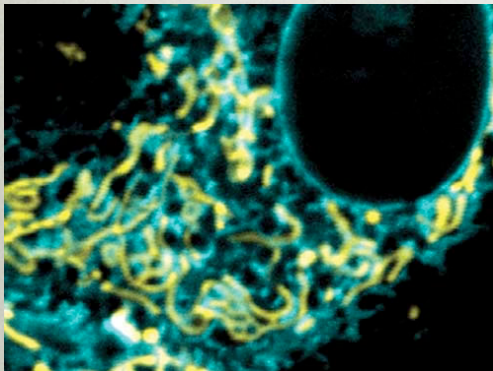
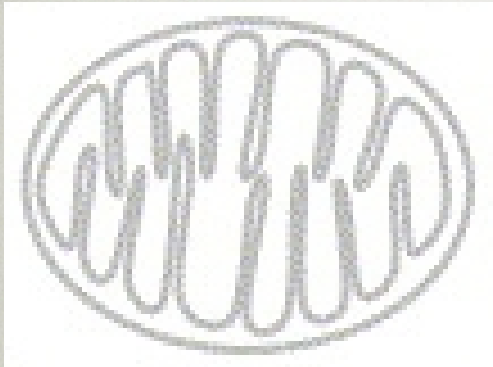


Bardeesy, N and RA DePinho. *Nature Reviews Cancer*, 2002.

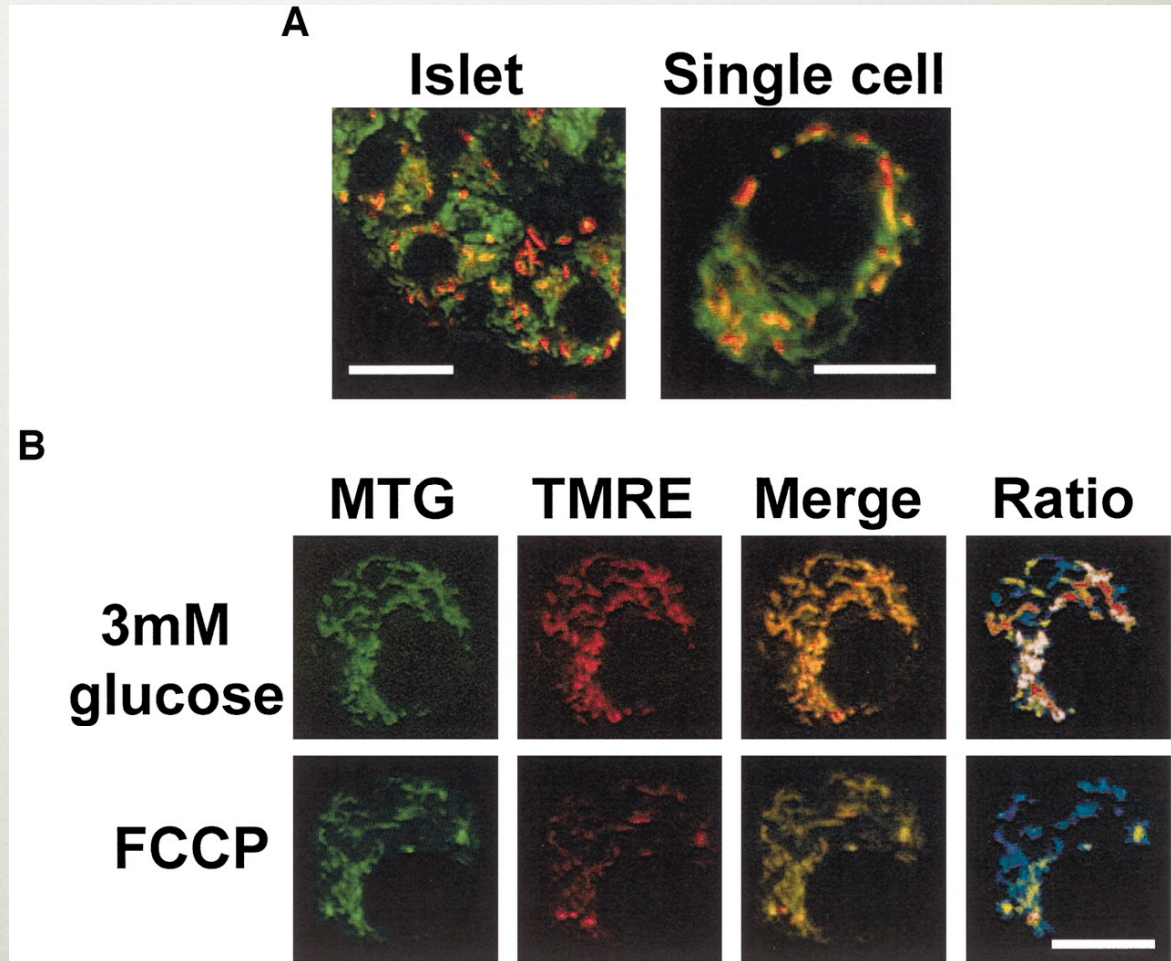
The Pancreatic β -Cell



Mitochondria



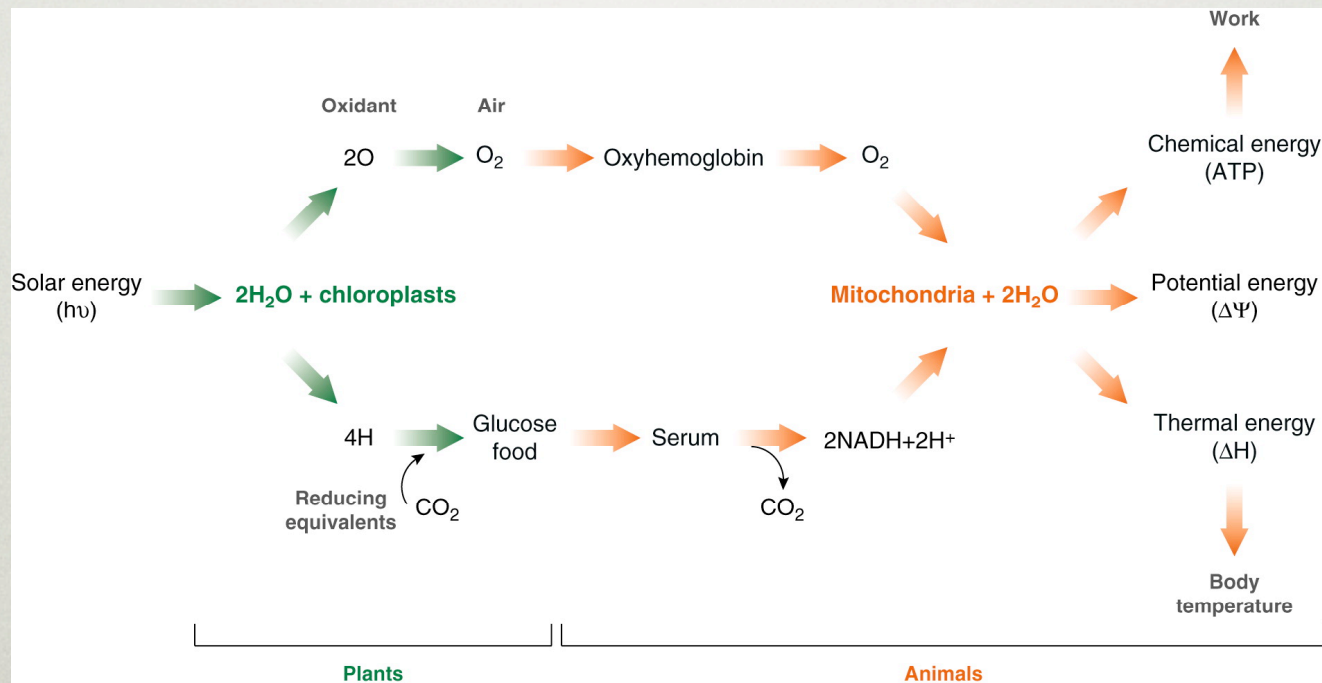
Piston, D, Vanderbilt University.



Wikstrom *et al.*, *Diabetes*, 2007.

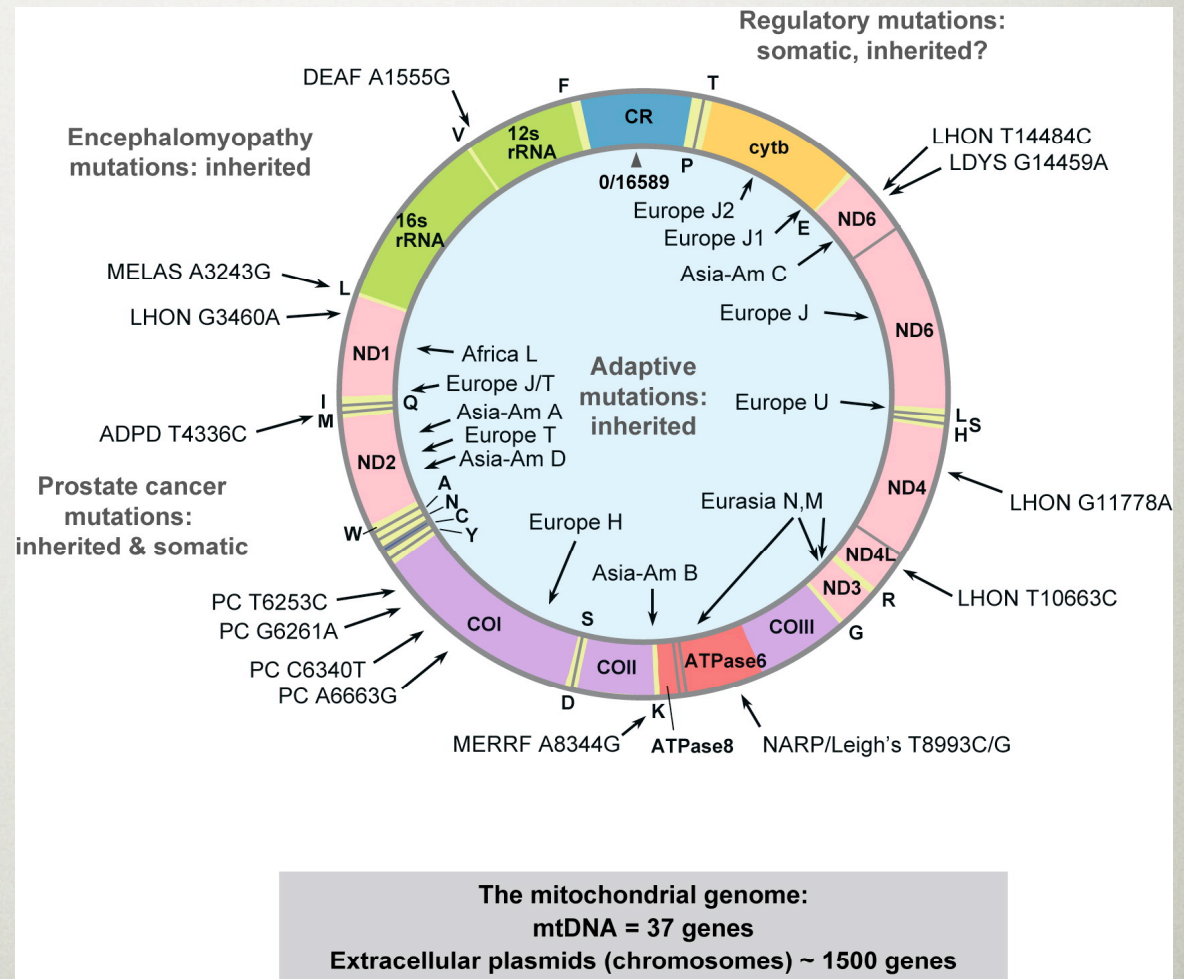
History of Mitochondria

- Eukaryotic ancestors engulfed or were infected by ancient bacteria ~ 2 billion years ago in symbiosis.
- Structure, energy, and information.



Mitochondrial DNA

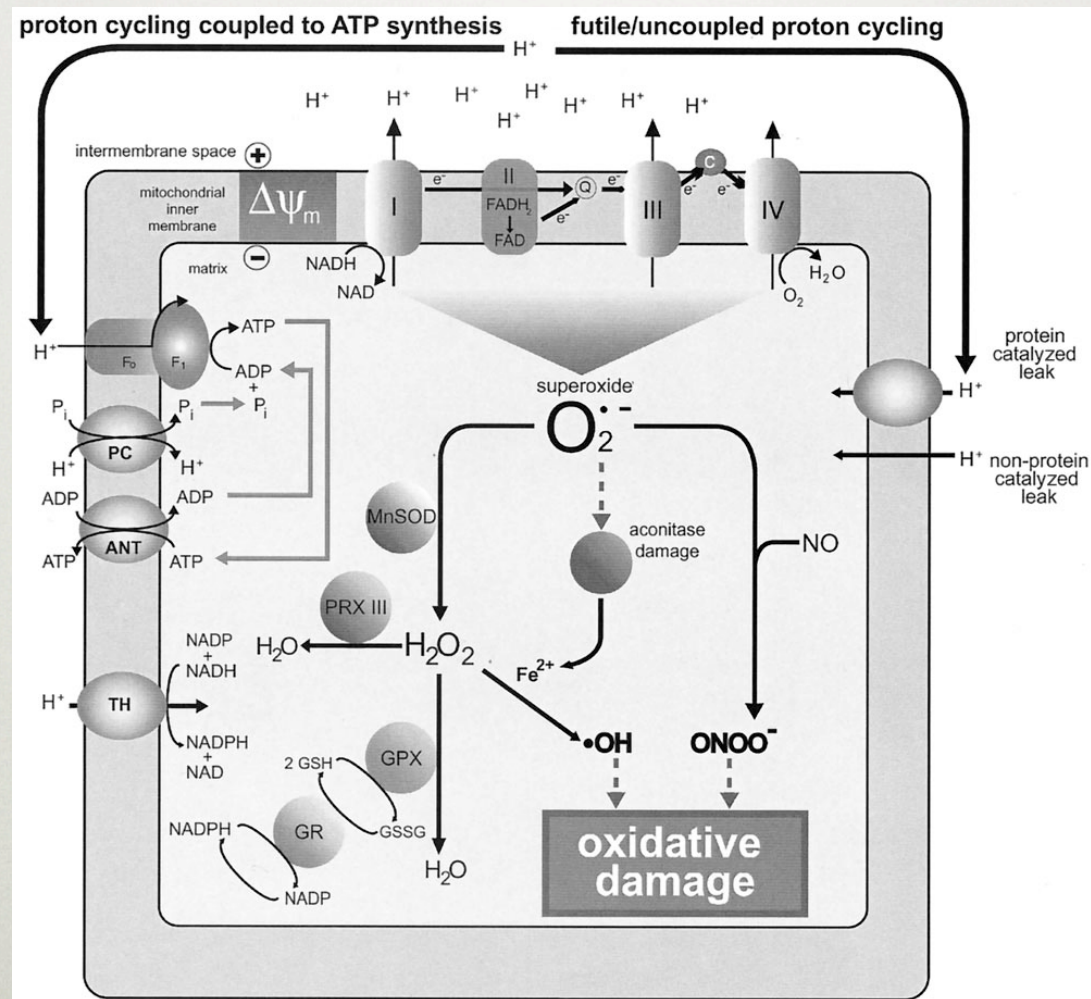
- 37 genes = 13 subunits of OXPHOS + 22 tRNA + 2 rRNA.
- ~ 2–10 mtDNA copies per mitochondria and 100's of mitochondria per cell.



mtDNA mutations

- Mutations → aging, mitochondrial dysfunction, diseases, cell death, etc.
- Mutation rate is 1–2 orders of magnitude higher than nuclear mutation rate.
- No recombination, so this high mutation rate is important in keeping mitochondria diverse, i.e., it is the adaptive engine.

Reactive Oxygen Species (ROS)



Green, K, MD Brand, and MP Murphy, *Diabetes*, 2004.

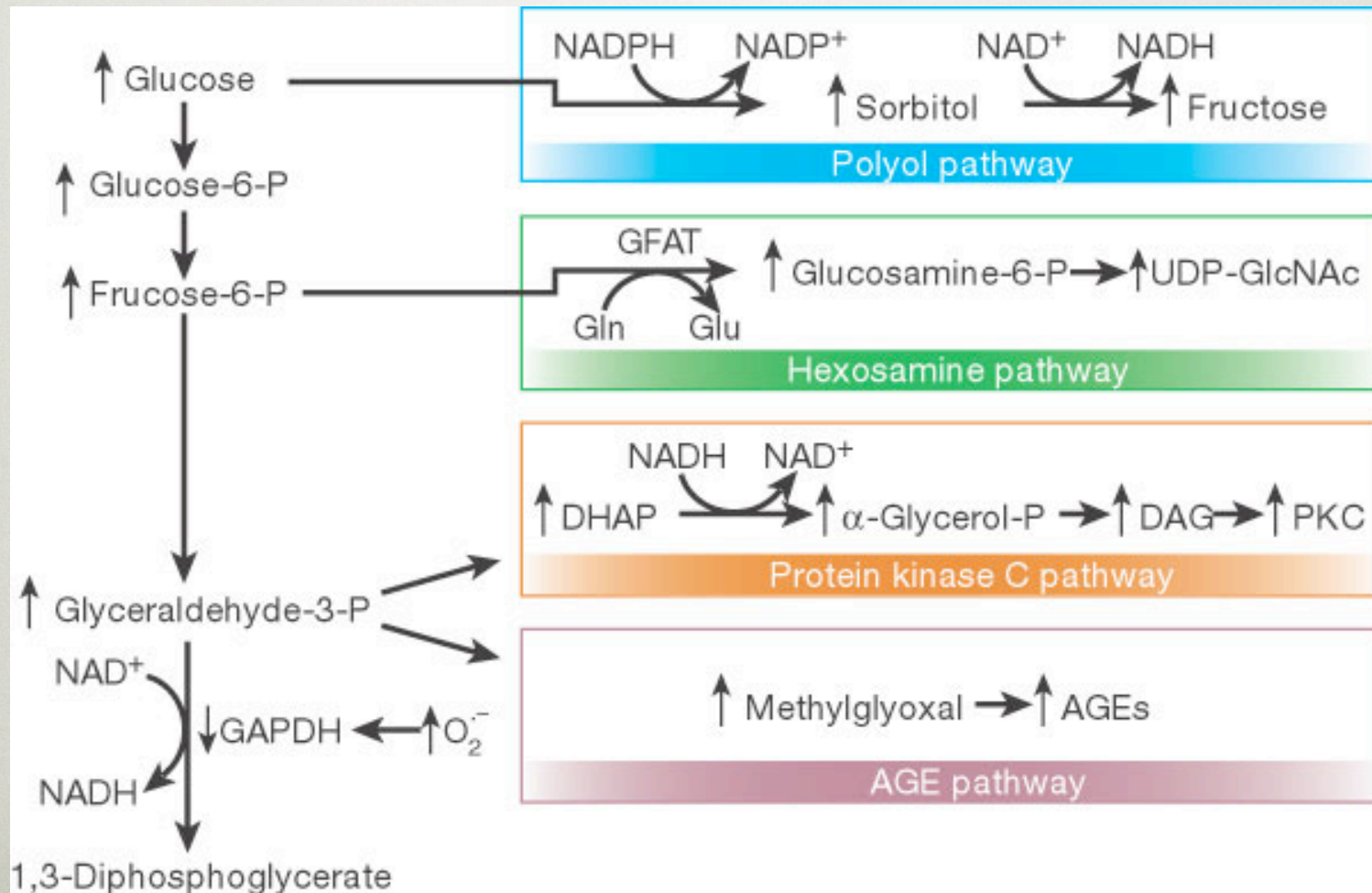
Banting Lecture 2004

The Pathobiology of Diabetic Complications

A Unifying Mechanism

Michael Brownlee

Diabetes, 2005.



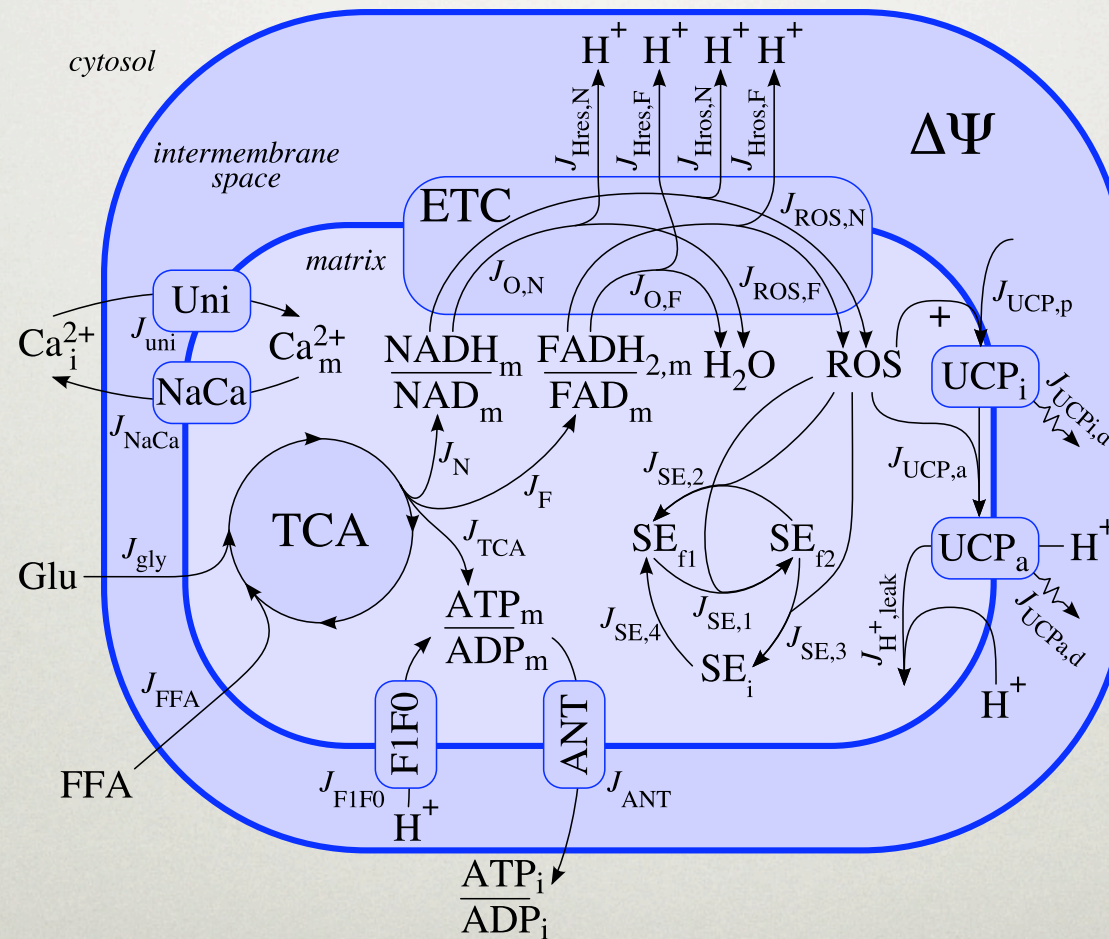
Background

- Reactive oxygen species (ROS) can cause oxidative damage, but also have important signaling functions.
- Uncoupling proteins (UCP) contribute to proton leak across the inner membrane, diverting protons from ATP production while decreasing ROS production.
- Mitochondrial ROS and UCP have been reported to play a key role in pancreatic β -cell function/dysfunction under various environmental conditions.
- Although there is evidence to support this, mechanistic details of ROS and UCP regulation have not yet been unraveled.
- Furthermore, experiments typically focus on effects from long-term exposure to nutrient levels, rather than the short-term responses.

Aims

- Develop a state-of-the-data mathematical model of beta-cell mitochondrial respiration, ATP synthesis, and ROS production/regulation in response to glucose and fatty acid stimulation. The model should be able to match existing experimental observations of UCP content and the proton leak rate.
- Use the model to test our current understanding of the system, and propose hypotheses related to short- and long-term perturbations in various environments.
- Use the model to predict an individual's insulin secretion rate and quantify β -cell function.

The Model System



The Model

- R Bohnensack, J Bioenerg Biomembr, **14**:45-61, 1982.
- D Pietrobon and SR Caplan, Biochem, **24**:5764-5778, 1985.
- **G Magnus and J Keizer, Am J Physiol, 273:C717-C733, 1997, 274:C1158-C1173 and C1174-1184, 1998.**
 - S Cortassa et al., Biophys J, **84**:2734-2755, 2003.
 - R Bertram et al., J Theor Biol, **243**:575-586, 2006.
- S Salinari et al., Am J Physiol Endocrinol Metab, **293**:E396-E409, 2007.

The Complete Model

$$\frac{dNADH_m}{dt} = \gamma (J_{Glu,N} + J_{FA,N} - J_{O,N} - J_{ROS,N})$$

$$\frac{dFADH_{2,m}}{dt} = \gamma (J_{Glu,F} + J_{FA,F} - J_{O,F} - J_{ROS,F})$$

$$\frac{dATP_m}{dt} = \gamma (J_{F1F0} + J_{TCA,Glu} + J_{TCA,FA} - J_{ANT})$$

$$\frac{dCa_m}{dt} = f_m (J_{uni} - J_{NaCa})$$

$$\frac{d\Delta\Psi}{dt} = (J_{Hres,N} + J_{Hres,F} + J_{Hros,N} + J_{Hros,F} - J_{H,atp} - J_{ANT} - J_{H,leak} - J_{NaCa} - 2J_{uni}) / C_m$$

$$\frac{dROS}{dt} = 2(J_{ROS,N} + J_{ROS,F}) - J_{SE,1} - 2J_{SE,2} - J_{UCP,a}$$

$$\frac{dSE_{f1}}{dt} = J_{SE,2} + J_{SE,4} - J_{SE,1}$$

$$\frac{dSE_{f2}}{dt} = J_{SE,1} - J_{SE,2} - J_{SE,3}$$

$$\frac{dUCP_i}{dt} = J_{UCP,p} - J_{UCP,a} - J_{UCP_d,i}$$

$$\frac{dUCP_a}{dt} = J_{UCP,a} - J_{UCP_d,a}$$

$$NAD_{tot} = NAD_m + NADH_m$$

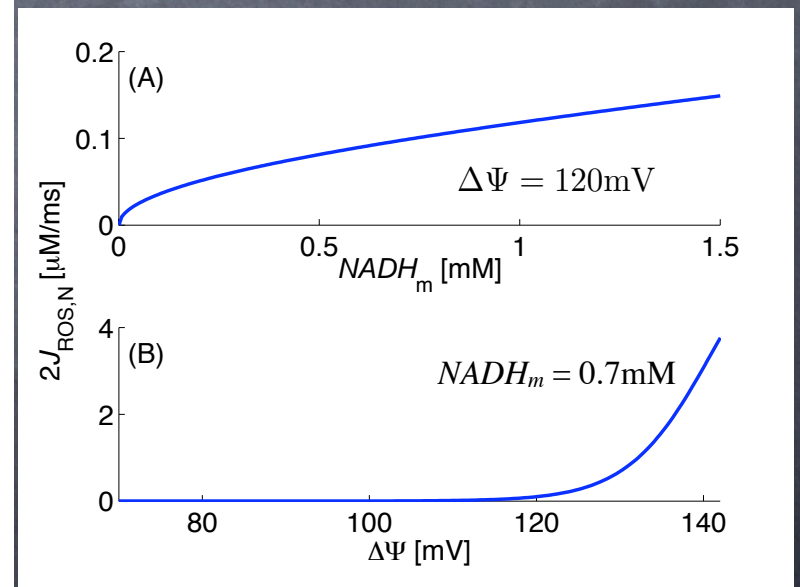
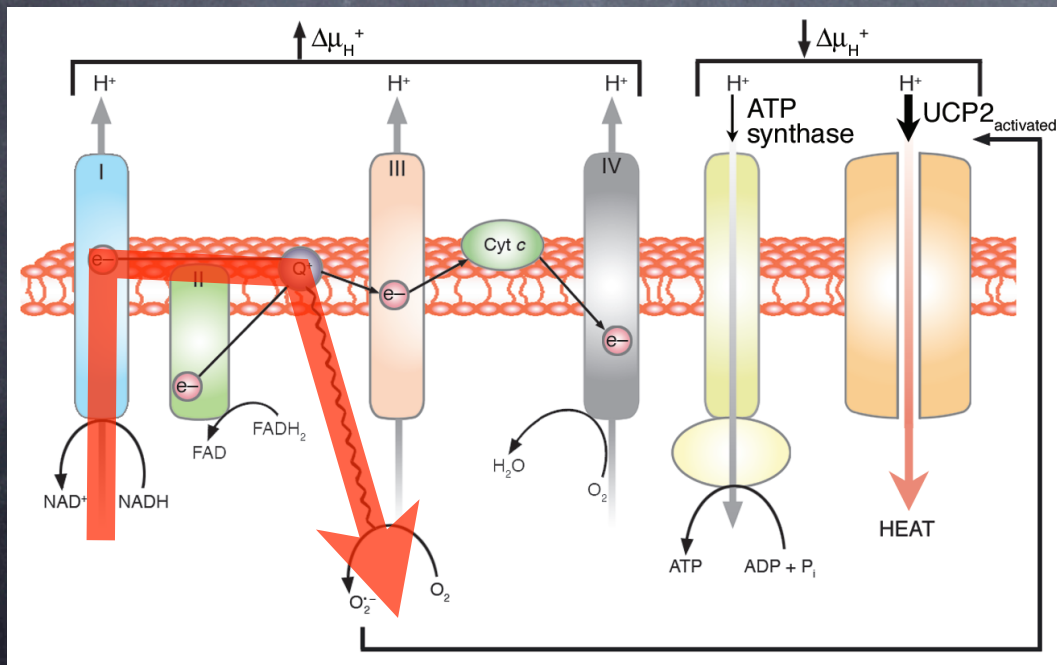
$$FAD_{tot} = FAD_m + FADH_{2,m}$$

$$A_{tot} = ADP_m + ATP_m$$

$$SE_{tot} = SE_{f1} + SE_{f2} + SE_i$$

ROS Production from NADH Oxidation

$$J_{ROS,N} = p_{15} \sqrt{\frac{NADH_m}{NAD_m}} \left(1 - \frac{1}{1 + e^{(\Delta\Psi - p_{16})/p_{17}}} \right)$$



Brownlee, *J Clin Invest*, 2003.

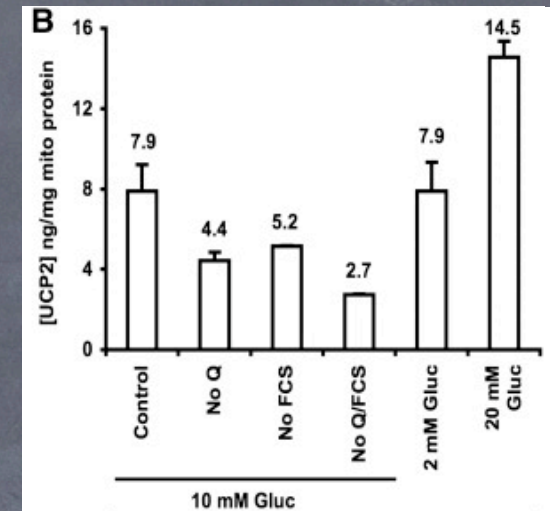
Uncoupling Proteins (UCP)

$$J_{UCP,p} = p_{23}ROS_{delay} + p_{24},$$

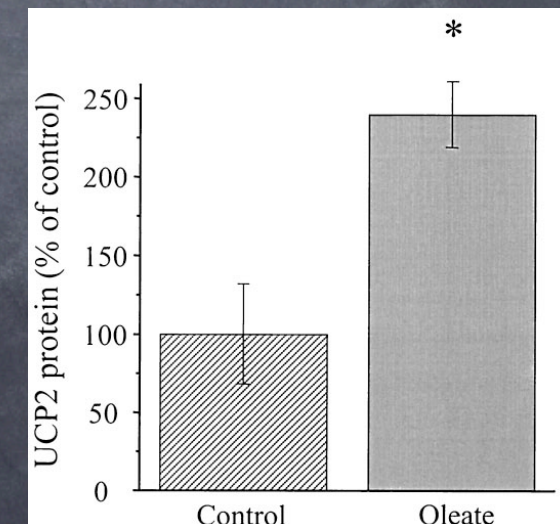
$$J_{UCP,a} = p_{25}UCP_iROS,$$

$$J_{UCP_{d,i}} = p_{26}UCP_i,$$

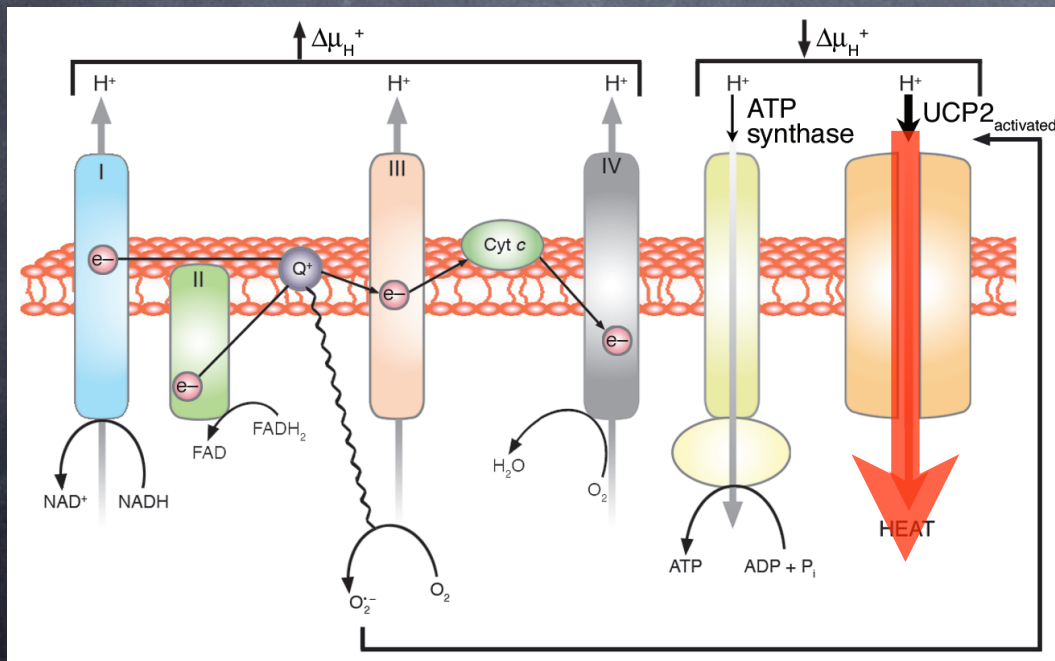
$$J_{UCP_{d,a}} = p_{26}UCP_a$$



Azzu *et al.*, *Biochim. Biophys. Acta*, **1777**:1378-1383, 2008.

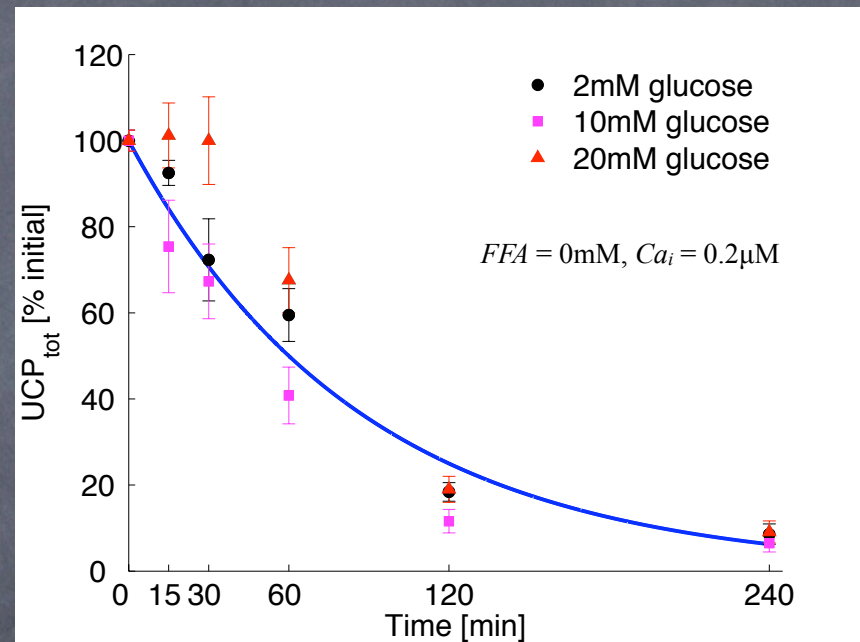
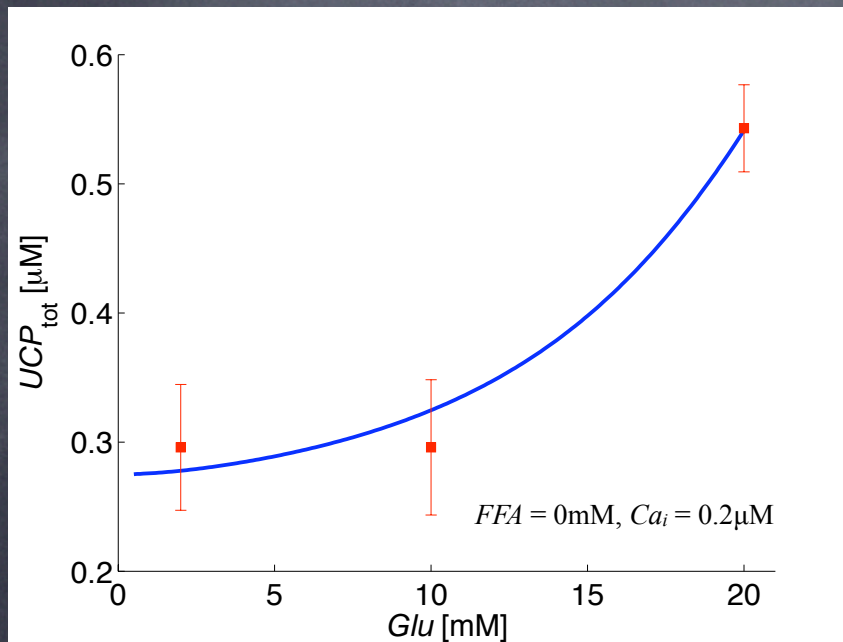


Lameloise *et al.*, *Diabetes*, **50**:803-809, 2001.



Brownlee, *J Clin Invest*, 2003.

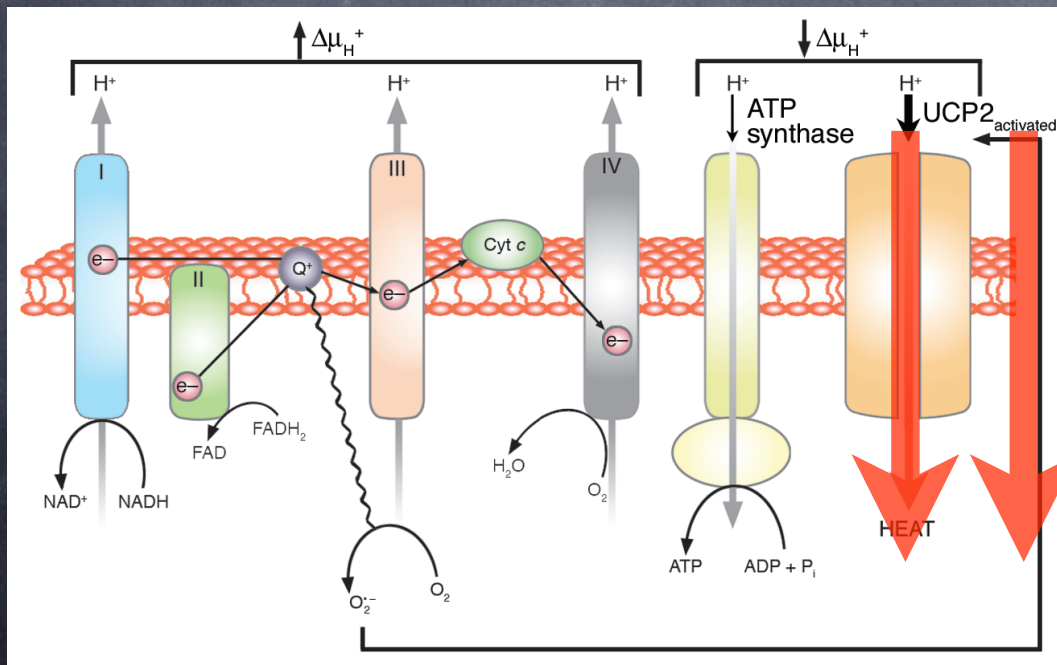
Uncoupling Proteins (UCP)



Data from Azzu *et al.*, *Biochim. Biophys. Acta*, 2008.

Proton Leak

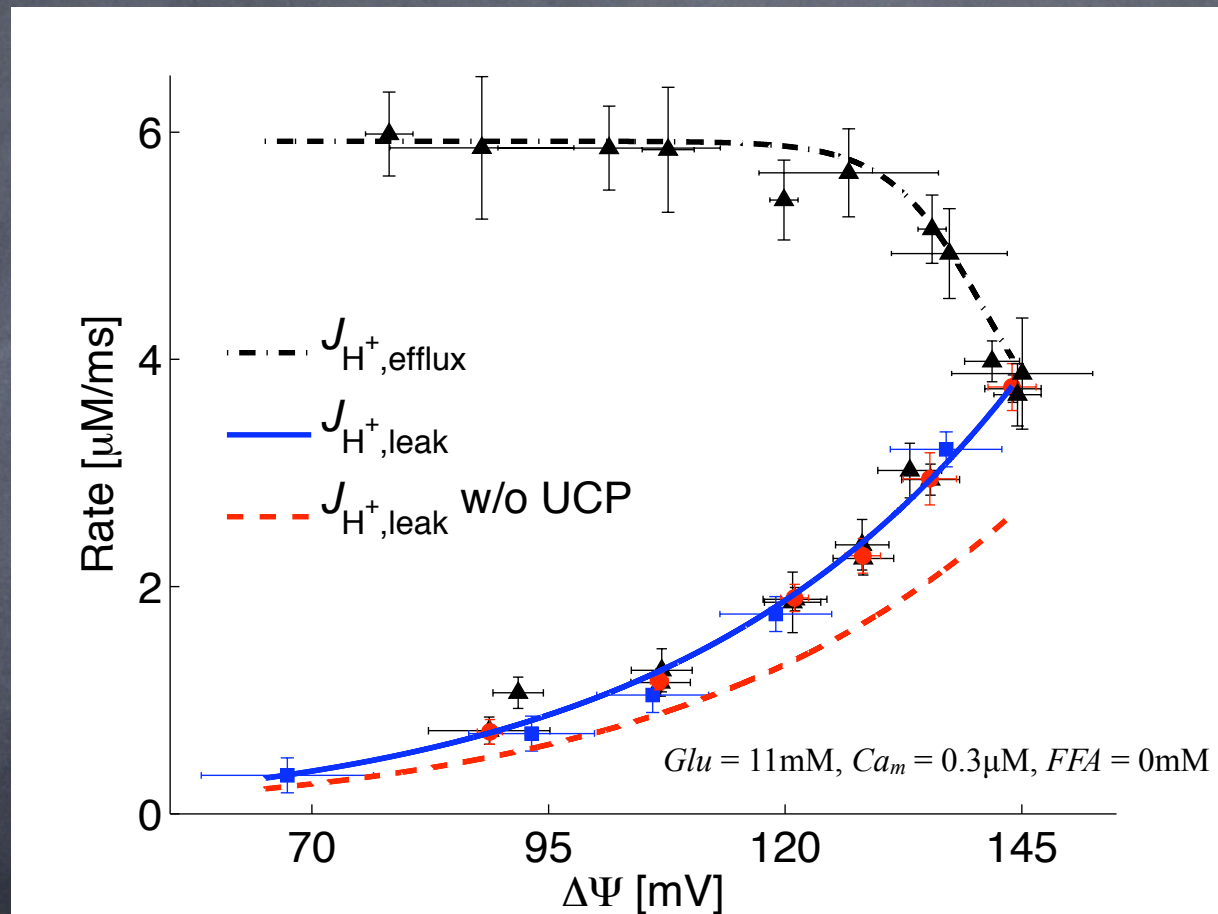
$$J_{H^+, leak} = \Delta\Psi e^{p_{41}\Delta\Psi} (p_{42} + p_{43}UCP_a)$$



Brownlee, *J Clin Invest*, 2003.

$\Delta\Psi$

The Nonlinear Proton Leak Rate

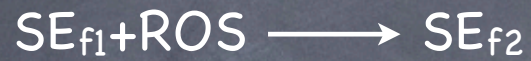


Squares: Echtay, KS *et al. Nature*, 2002.

Diamonds: Affourtit, C and MD Brand. *Biochem. J.* 2006.

Circles: Affourtit, C and MD Brand. *Biochem. J.* 2008.

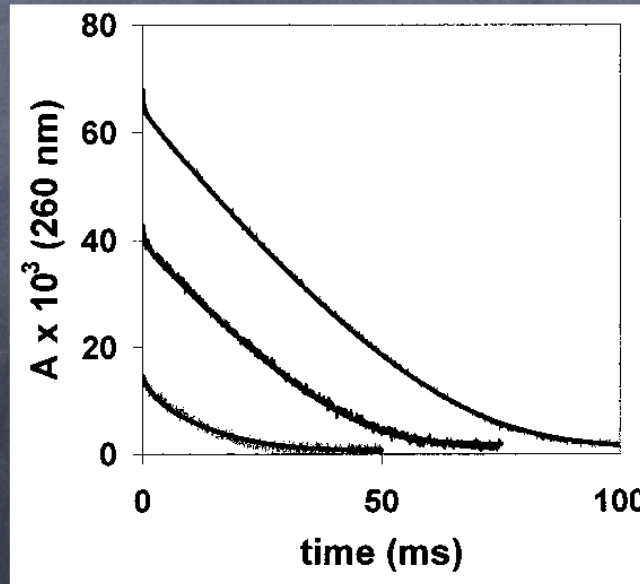
Scavenging Enzymes



$$J_{SE,1} = p_{34} SE_{f1} ROS,$$

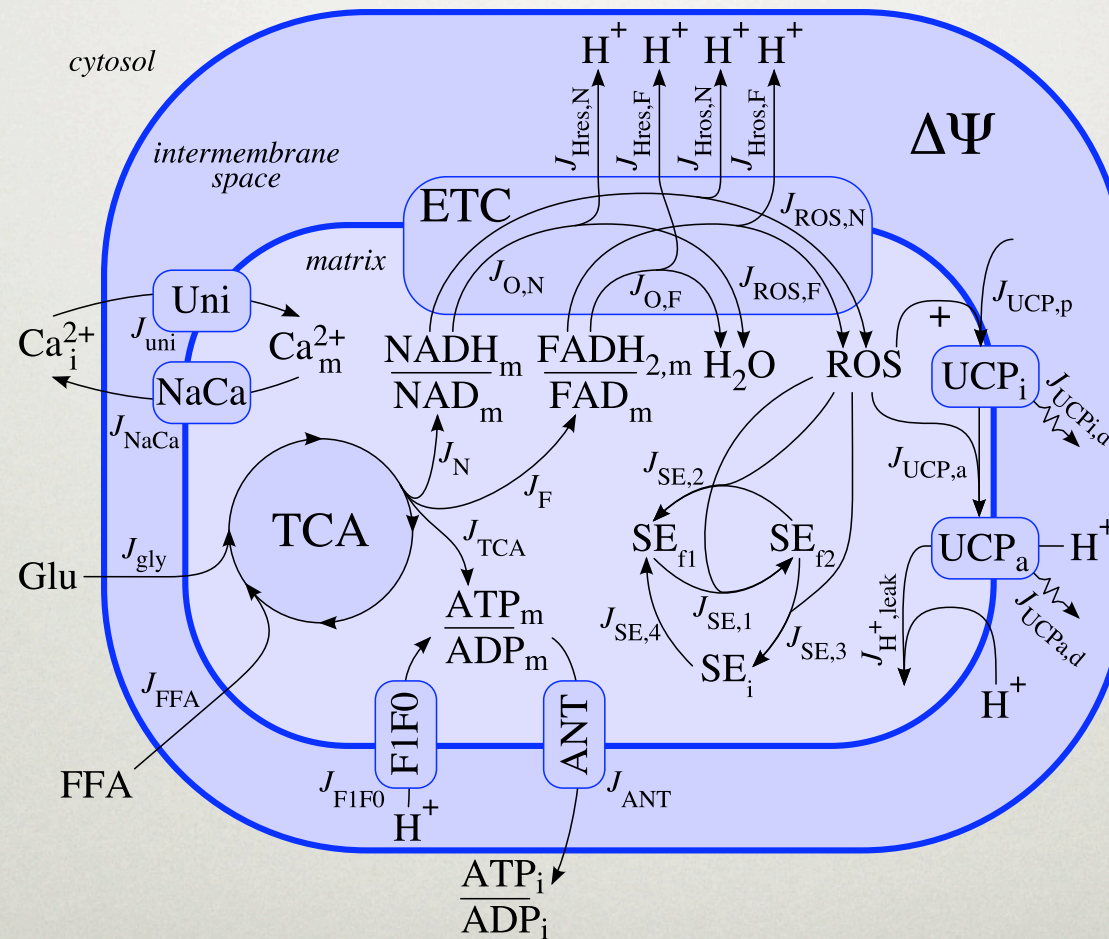
$$J_{SE,2} = J_{SE,3} = p_{35} SE_{f2} ROS,$$

$$J_{SE,4} = p_{36} SE_i$$

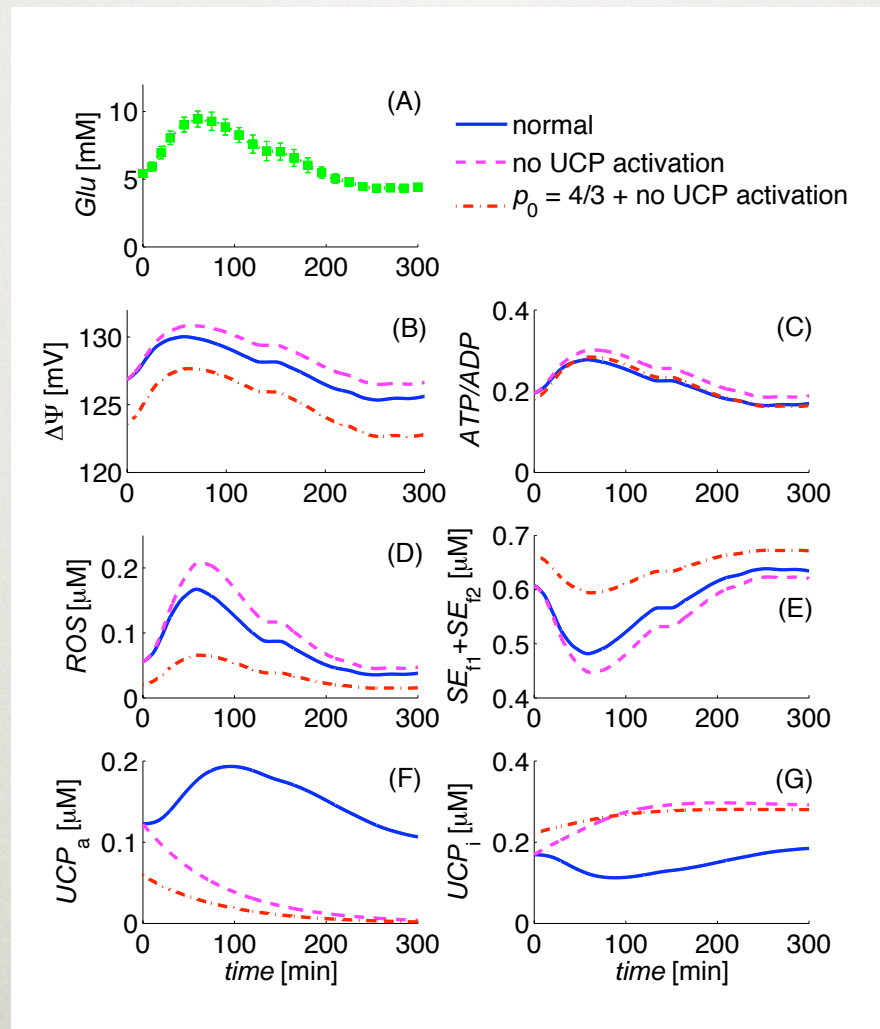


Hearn, A et al., *Biochem*, 2001.

The Model System



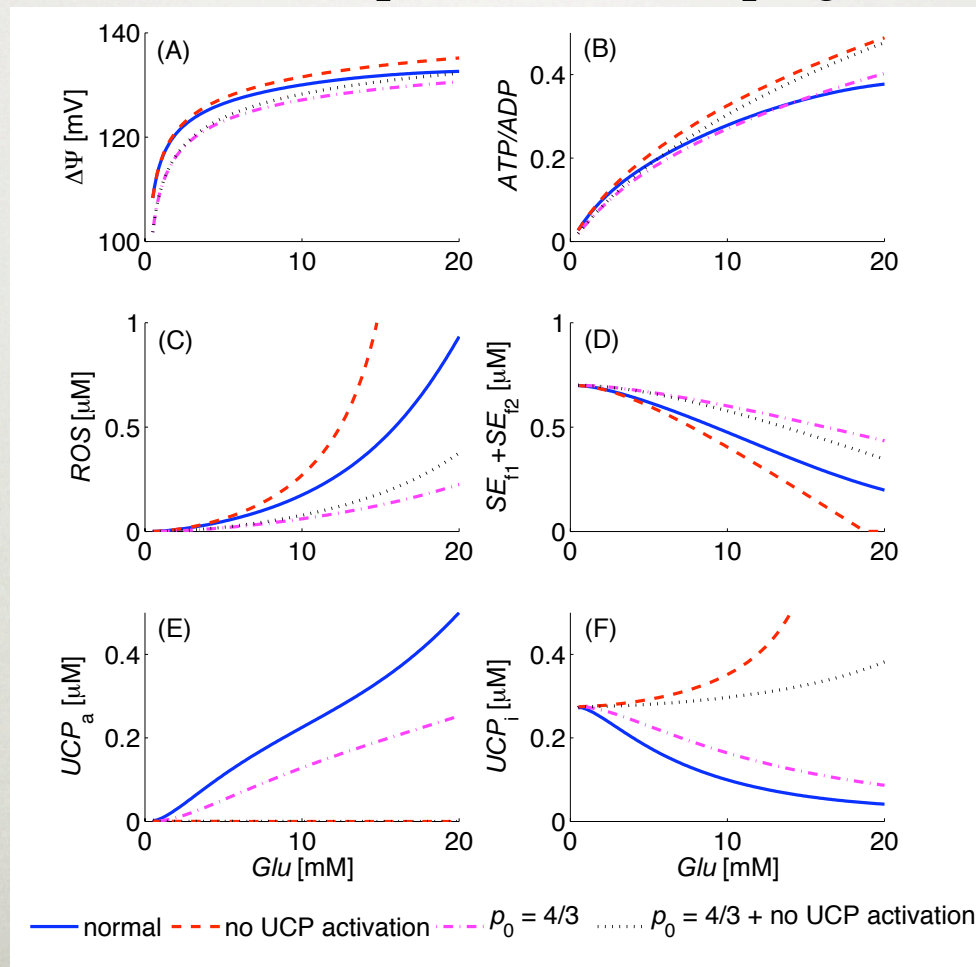
Short-term responses to glucose: ROS signaling and the ATP/ADP response are improved with acute inhibition of UCP activity.



$FFA = 0\text{mM}$, $Ca_i = 0.2\mu\text{M}$
 Profile from Breda, E *et al. Diabetes*, 2001.

Long-term responses to glucose:

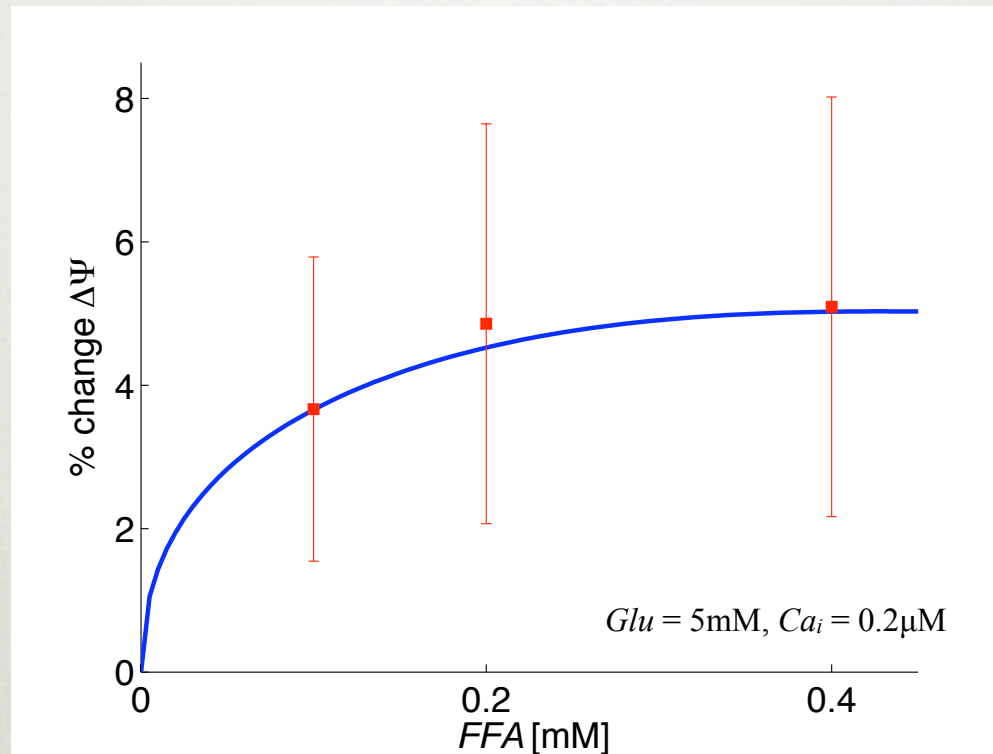
Model predicts blocking UCP activation causes sustained increases in ROS levels, and thus oxidative stress, but distributing the metabolic load by increasing the mitochondrial density (p_0) improves the ATP/ADP response while keeping ROS levels low.



FFA = 0mM, $Ca_i = 0.2\mu$ M

Short-term responses to fatty acids:

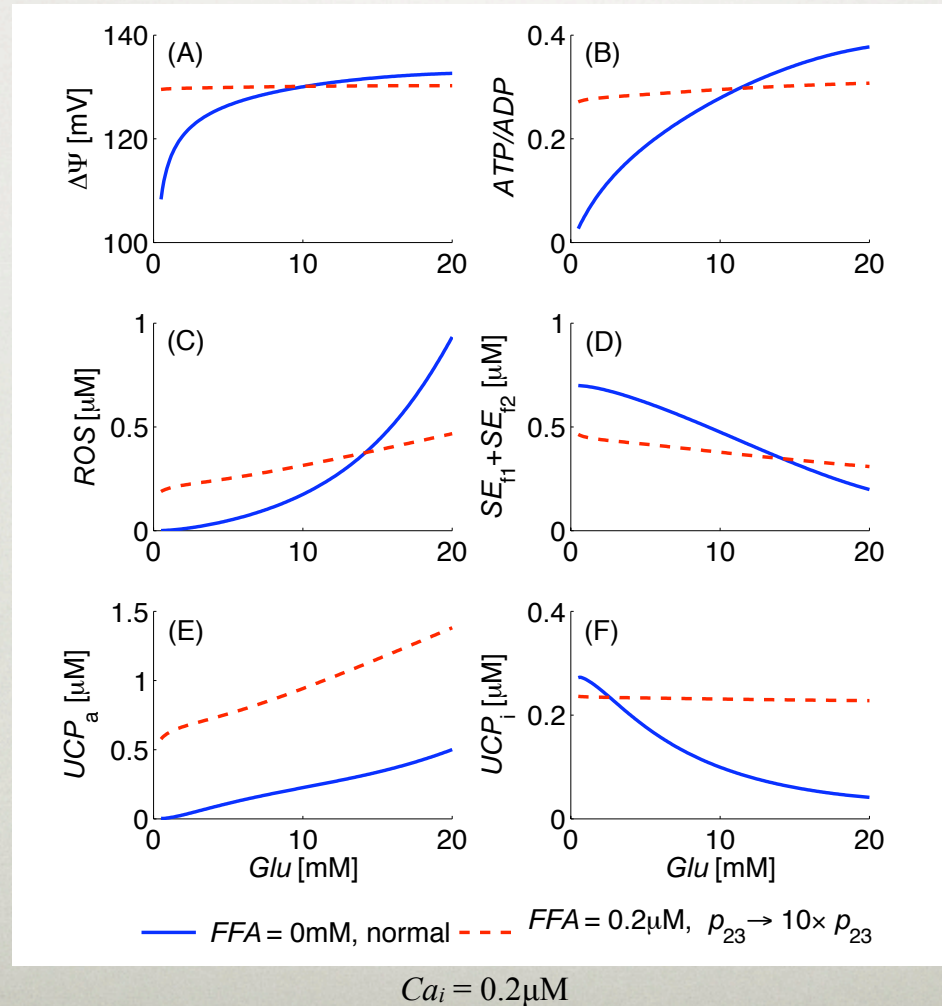
Addition of fatty acids causes an increase in the membrane potential (and the other mitochondrial variables) that saturates for higher fatty acid concentrations



Data from Carlsson, C *et al.* *Endocrinology*, 1999.

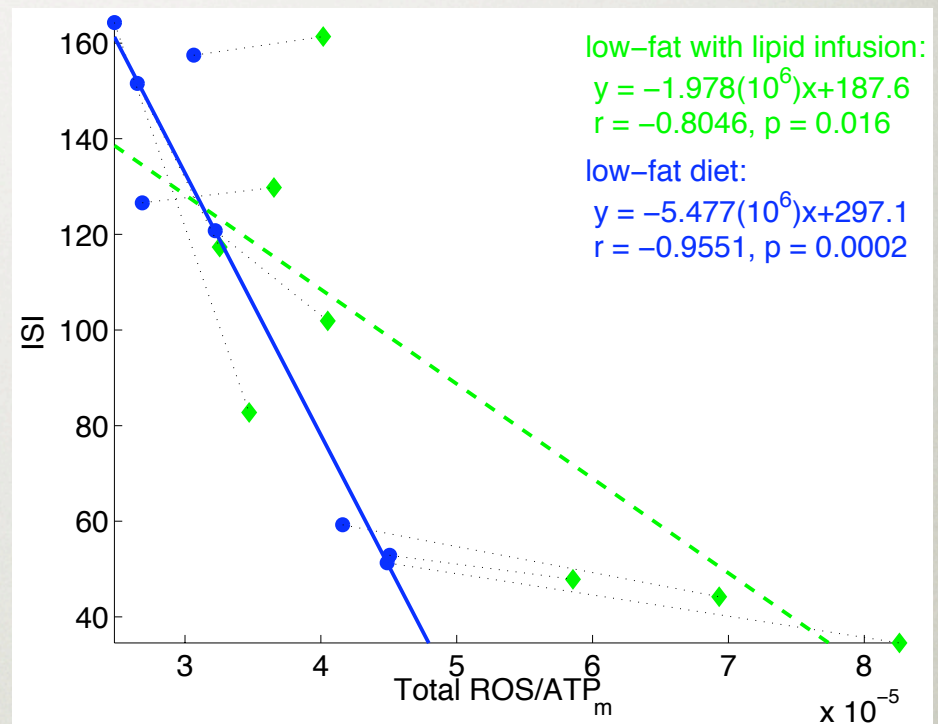
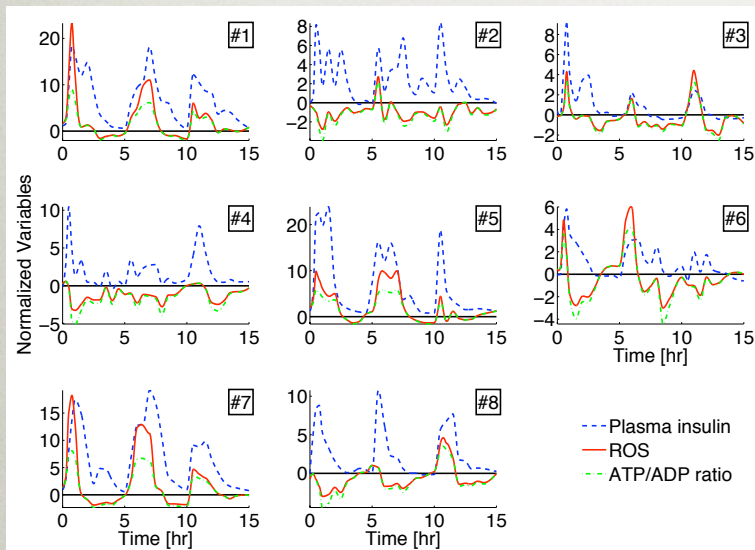
Long-term responses to fatty acids:

Model simulations suggest FFA could cause the experimentally observed crossover effect (i.e., an elevated basal insulin secretion, but decreased GSIS) by increasing the ROS-dependent UCP-production signal (model parameter p_{23}).



Clinical Applications?

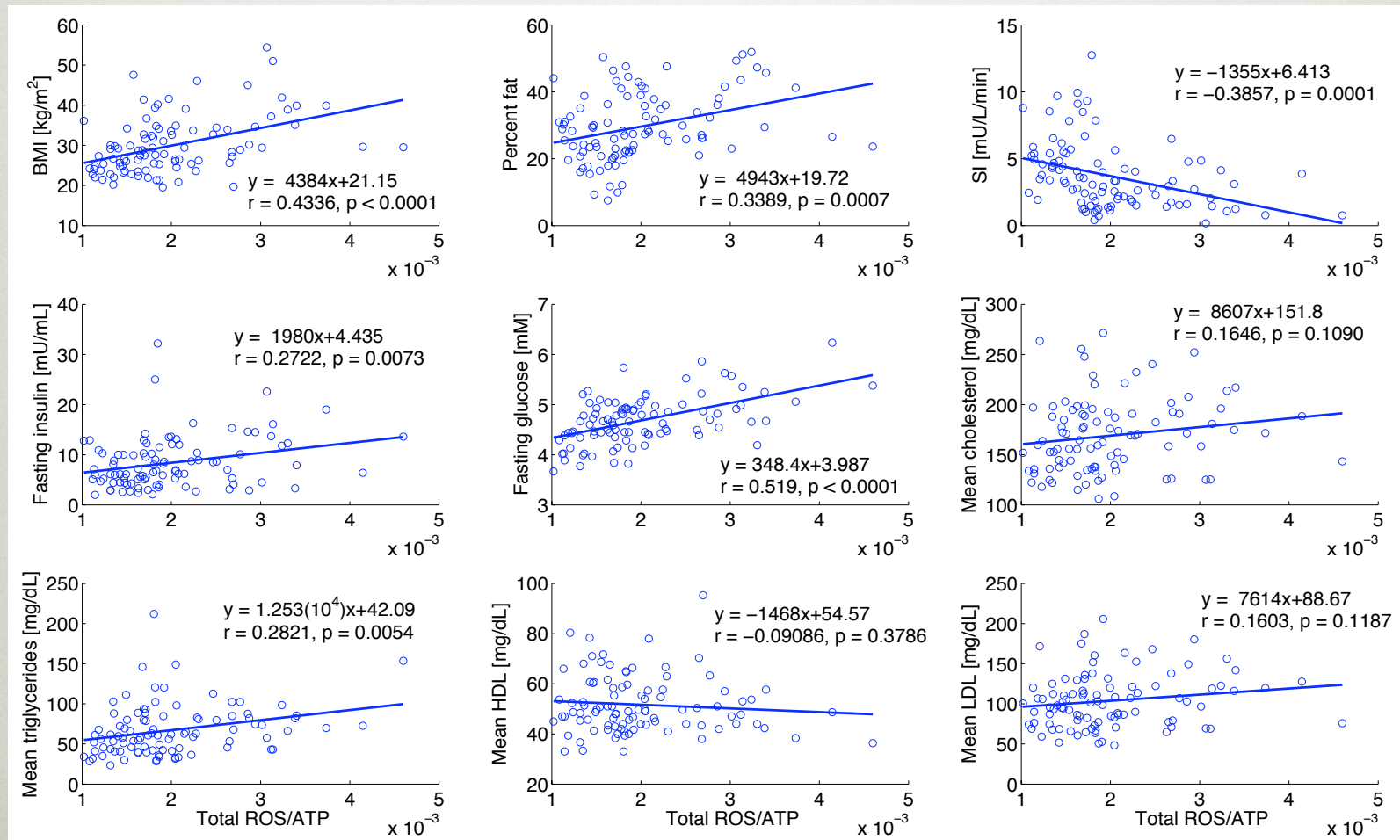
Diet Study 1: Eight overweight, mixed-ethnicity subjects.
Correlation between ISI and total ROS/ATP.



Data from Knuth, ND *et al.* *J Appl Physiol*, 2009.

Clinical Applications?

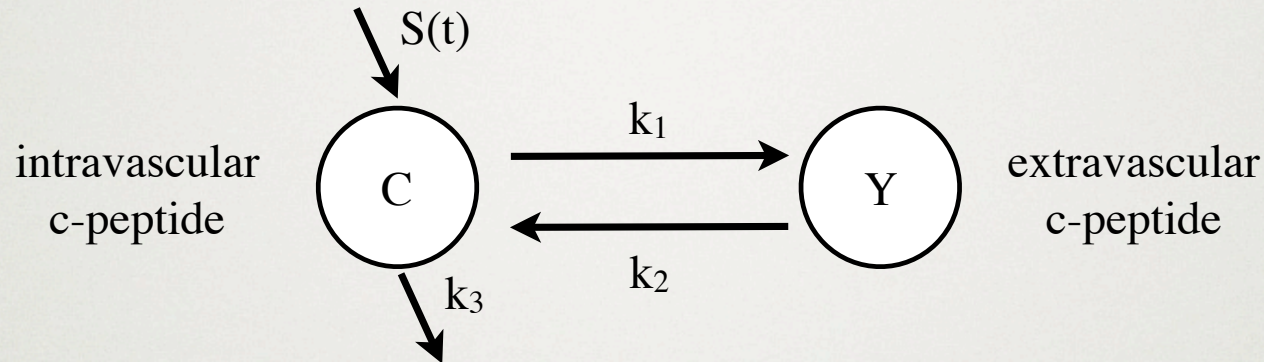
Diet Study 2: 102 nondiabetic, African-American subjects.
Correlations between several measures and total ROS/ATP.



Data from Periwal, V *et al.* *Am J Physiol Regul Integr Comp Physiol*, 2008.

A clinical application:

Our model may be useful in predicting the c-peptide and insulin secretion rates and quantifying β -cell function in an individual.

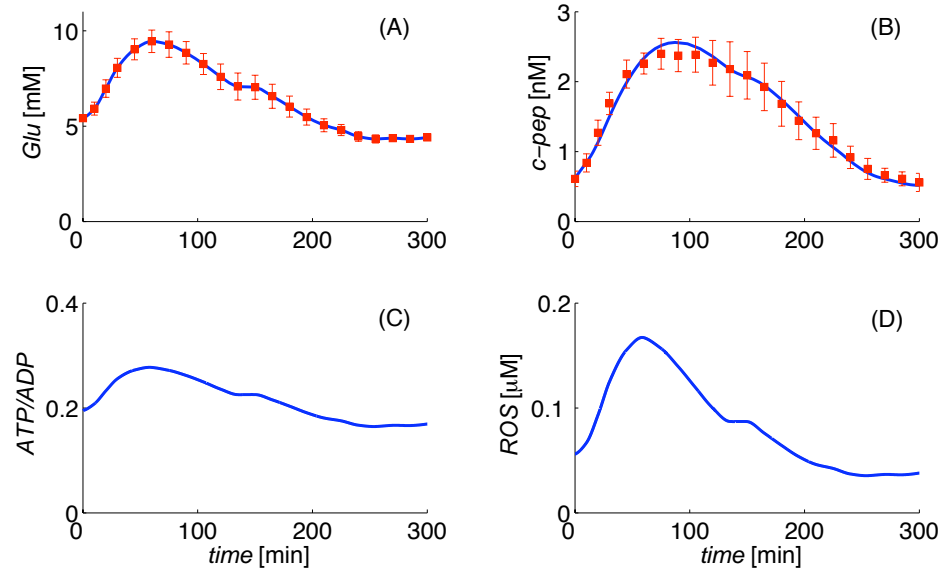


From Eaton, RP *et al. J. Clin. Endocrin. Metab.* 1980:

$$\frac{dC(t)}{dt} = -(k_1 + k_3)C(t) + k_2Y(t) + S(t)$$

$$\frac{dY(t)}{dt} = k_1C(t) - k_2Y(t)$$

$$S(t) = k_a \frac{ATP}{ADP} + k_r ROS - k_b$$



Profile from Breda, E *et al. Diabetes*, 2001.

FFA = 0mM, $Ca_i = 0.2\mu\text{M}$

Summary

- The model is consistent with a number of experimental observations, and it is capable of predicting mitochondrial responses to nutrient inputs (glucose and fatty acids).
- It provides a tool to test the current understanding of a complex system, as many details of the autoregulation of ROS via UCP control have not yet been fully unraveled in the experimental literature.
- Model predictions provide testable hypotheses; e.g. increasing mitochondrial density and inhibiting UCP activation may increase GSIS while decreasing oxidative stress.
- The model may have useful clinical applications; e.g. the c-peptide secretion rate model, if standard parameter values can be identified and compared to on an individual basis.

Future Work

- Address limitations of the current model; e.g. consider dynamic antioxidant content, other endogenous UCP activators, and other pathways of proton leak.
- Apply the c-peptide secretion rate model to a large data set to try to identify standard parameters.
- Extend the model to mitochondria in other tissues. This may be useful in suggesting holistic metabolic therapies given the systemic function that mitochondria serve.



Acknowledgments

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Collaborators:
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Kristina Rother

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